The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 2 JULY, 1936 NUMBER 4

LABORATORY STUDIES IN RHEUMATOID ARTHRITIS

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There is, perhaps, no subject to which the medical profession of today is giving as much attention as to chronic arthritis. This disease, long one of the mysteries of medicine, is beginning to yield many of its secrets. For many years this disease was regarded by the profession and the laity as hopelessly chronic, but this unfortunate situation is rapidly being corrected and today there are arthritis clinics in nearly every large city of the country. The clinical laboratory has played no small part in the investigations which have brought about this change in the prognostic point of view.

About 75% of all the cases of chronic arthritis are of the rheumatoid type (synonyms—chronic infectious arthritis, atrophic arthritis, arthritis deformans). Twenty per cent are of the osteoarthritic type (synonyms—hypertrophic arthritis, degenerative arthritis). The remaining 5% are of the gonorrhoeal, traumatic, tubercular, and luetic types. These ratios may be almost reversed in some parts of the country due to the decrease of rhumatoid arthritis in the warm, dry climates.

The belief that rheumatoid arthritis is infectious in origin is quite generally accepted. The exact infectious agent, however, is not so generally agreed upon. Some people believe that there is a direct infection of the joint, while others believe that the joint condition is a result of an allergic response from a distant focus. Re-

cent investigations point toward the streptococcus as the infectious agent in the majority of cases, and these investigations have also shown that there is a direct infection of the joint in well established cases.

Streptococci have been recovered from the blood, joint fluid, lymph nodes and subcutaneous nodules in a higher percentage of cases of rheumatoid arthritis than any other organism, as shown in Table I.

TABLE I

BACTERIOLOGY OF RHEUMATOID ARTHRITIS

Source	Authors	Year	% Pos. for Streptococci
Lymph glands	Rosenow[1]	1914	59%
Lymph glands	Poston[2]	1929	59%
Subcutaneous nodules	Clawson & Wetherby[3]	1932	71%
Joint fluid	Cecil, Nichols & Stainsby[5]	1931	67%
Joint fluid	Gray & Gowen[4]	1931	62.5%
Blood cultures	Moon & Edwards[5]	1917	26%
Blood cultures	Cecil, Nichols & Stainsby[6]	1931	62%
Blood cultures	Klugh[7]	1931	71%
Blood cultures	Gray & Gowen[8]	1931 (Jan.)	67.6%
Blood cultures	Gray & Gowen[4]	1931 (Nov.)	58%
Blood cultures	Gray, Fendrick & Gowen[9]	1932	60%

For the most part, the organisms recovered from the various sources have been either an alpha or an alpha prime streptococcus. These organisms do not appear to represent a specific strain, though falling in a fairly well-defined group. Most of the strains grow very poorly on primary planting and produce a faint green discoloration on blood agar.

Isolation of streptococci from the blood has, for the most part, been successful only to those who have used a double strength heart infusion broth enriched with a carbohydrate. As most of these organisms do not grow well in free oxygen, it is necessary to use large amounts of broth in deep bottles or flasks, or create oxygen tension by the addition of gelatin. It is also necessary to inhibit or destroy the native complement in the patient's serum by culturing the clot alone or absorbing or neutralizing the complement by various measures. As the organisms produce large amounts of acid it is desirable to use a well buffered medium. Great care must also be used in the selection of fresh beef hearts and no pressure used in sterilizing the finished product.

The culture method which I have used and which has been described by myself and co-workers[8, 9] will be reviewed in detail as follows:

Materials

Syringes and Needles for Taking the Blood: Twenty c.c. Luer syringes and 19 gauge needles are placed in glass containers and sterilized by dry heat at 180° C. for two hours.

Tubes for Collecting the Blood: Test tubes, 6 inches by 1 inch, are plugged with non-absorbent cotton, capped and dry sterilized at 180° C. for two hours.

Culture Bottles: Four ounce Pyrex bottles, 2 inches in diameter, are plugged with cotton and cheese cloth, 10 to 15 grams of marble are placed in each bottle and they are dry sterilized at 180° C. for two hours.

Arnold Sterilizer: A type should be used in which no pressure is developed.

Hydrogen-ion Set: The La Motte type is used with a pH scale of 6.8 to 8.4, using phenol red as an indicator.

Pipettes: Tubes of even bore, three-eighths inch in diameter and 14 inches in length, are used. One edge should be sharp to facilitate breaking up the clot.

Peptone: "Wittes" peptone has been found the most satisfactory.

Sodium Chloride and Dextrose: Chemically pure products should be used.

Gelatin: Sheet (Super X) is advised.

Beef Heart: This must be fresh from the abattoir.

Preparation of Media

Fresh beef heart is freed from fat and fibers, ground finely in a meat chopper, and infused in the ice box overnight. The next morning the infusion is warmed to 20° C. to 25° C. and squeezed through a flannel bag. The filtrate is boiled slowly for one hour and filtered through paper. It is made up to volume, 1.5% peptone, 0.5% sodium chloride, 1% dextrose and 1% gelatin added and the ingredients dissolved by warming in the Arnold sterilizer for 20 to 25 minutes. Following this it is titrated to pH 8, heated in the Arnold sterilizer for one hour, filtered through paper and re-titrated. If the pH has fallen below 7.8 it should be re-titrated to that figure. Approximately 50 to 60 c.c. of media is placed in each of the sterile bottles (the depth of the media should be approximately 1.25 inches), and sterilized in the Arnold for 30 minutes on three successive days. At the end of three days it is again titrated and if the pH is 7.6 to 7.8, is satisfactory for use. The media is incubated for several days and, if sterile, is ready for use.

Preparation of Blood Agar Plates

The base used for the plates is a 1.5% agar sugar-free heart infusion medium titrated to a pH of 7.4. This is stored in 4 ounce Pyrex bottles in amounts of 100 c.c. These are heated, cooled to 45° C. and 5 c.c. of citrated human, rabbit or sheep blood added. 10 c.c. of medium is used for each plate.

Cultural Technique

The patient's arm is prepared by applying two coats of iodine, the second coat being applied after the first has dried. Twenty c.c. of blood is drawn from the vein and placed in the dry sterile test tubes (10 c.c. in each). It is allowed to clot, and placed in the ice box overnight. The next morning the tubes are covered with oiled silk, shaken to loosen the clots, and centrifuged to separate the serum. The serum is taken off with a sterile pipette, and the clot broken up with the same pipette. Transference of the clot is then made to a culture bottle, shaken thoroughly and placed in the incubator. The cultures are examined every 24 hours for cloudiness or discoloration.

Growth Characteristics

A growth of streptococci found in rheumatoid arthritis usually shows a diffuse cloudiness with a dark brown discoloration of the media. The only other organisms we have encountered that show this color change are pneumococci and diphtheroid organisms. Staphylococci, beta streptococci and B. subtilis usually show a black or red discoloration. Stained specimens of young streptococci show a tendency toward long chains. Growths more than 24 hours old are usually short chained, and sometimes of diplococcic form. A satisfactory growth usually develops on a streaked blood agar plate in 24 to 48 hours. However, a blood broth tube is also seeded, since an occasional strain is found that will not grow in free oxygen, and it is necessary to sub-culture for several generations before a suitable growth is obtained.

Description of Organisms

Alpha prime colonies are grayish and oval. There is a small amount of methemoglobin formed, and after 24 or 48 hours incubation, a light ring of incomplete hemolysis appears. This hemolysis is slight, but may be readily seen with the hand lens. After removing the colony from the blood agar, a definite area of hemolysis is left in the medium. The hemolytic tendencies of this organism may become more pronounced on successive transplants.

Alpha colonies are grayish green, raised and pointed. They show definite production of methemoglobin and no zone of hemolysis. Even after many transplants, they never appear to become hemolytic.

In broth cultures most strains grow in long chains until the maximum growth is obtained, then they may break up in short chains or even diplococci. This happens very readily in carbohydrate media. The organisms are Gram positive, slightly lancet, shaped, bile insoluble, and do not ferment inulin.

A recent review of the blood cultures for streptococci, Table II, shows that there is considerable variation in positive findings at different stages of the disease.

TABLE II

BLOOD CULTURES FOR STREPTOCOCCI

	No. Cases	Cultures	Negative	Positive	Pos. Cases
Rheumatoid Arthritis					
(Early)	92	137	77	60	65%
(Established)	63	106	83	23	36%
(Advanced)	45	65	53	12	26%
Osteoarthritis	79	95	95	0	0%
Arthralgia	89	108	101	7	8%

It is of significance that the rheumatoid arthritis cases yielded a high percentage of positive cultures, and the osteoarthritis cases no positive cultures. In this table there is also shown a group called "arthralgia," which is distinguished from arthritis by a lack of joint swelling and a normal sedimentation rate. In all other respects they were similar, i.e., foci of infection associated with pain and stiffness in the muscles and joints. These patients were apparently in a pre-arthritic state, and possibly would have gone on to an arthritic condition without appropriate treatment. Therefore, it is not surprising that 8% of these cases showed positive cultures and that 16% showed agglutinins for streptococci to a high titre (Table III).

A small percentage of staphylococci and diphtheroids also appeared in the cultures and were considered as contaminants. Future investigations will probably show, however, that these so-called contaminants are of some significance because of the regularity of appearance in some cases.

Joint fluid cultures taken on patients during this study show that only in the established and advanced cases are positive cultures found. No positive cultures were found in the early cases. This is of some significance in showing that joint symptoms in early cases

of arthritis may be allergic, but in well established cases there is an actual joint infection.

In years past any positive blood culture was viewed with great concern, but one must remember that comparatively crude methods were used, and only the more virulent organisms were found. With the better methods of blood culturing in vogue today, many of the avirulent organisms discharged from a focus of infection are detected. This is unquestionably a bacteriemia, and is of significance only in showing the relationship of a focus to the disease in question.

Cultures made of various foci by Wood, Jensen and Post[10] show that streptococci predominate in 86%. Electrophoresis determinations made on these cultures from arthritic patients show a characteristic zone of electrical charge. Cataphoresis studies made on these organisms with the patient's serum show a definite relationship between the antibodies present and homologous bacteria. This is rather important evidence in showing that streptococci in a focus are in contact with the blood stream, and that the host attempts to combat these organisms by forming antibodies. If, for any reason, the antibodies formed are not of sufficient strength to control the organisms, a bacteriemia results as shown by the high percentage of blood cultures in the early cases, and in time a joint infection exists as shown by the positive joint fluid cultures in the established cases.

Agglutination Tests

Nichols and Stainsby[11] were the first authors to use this important diagnostic test. Later, other observers confirmed the finding of agglutinins in the blood of patients with rheumatoid arthritis. In normal and osteoarthritic patients agglutinins were absent or present in low titre. Cecil and his co-workers[6] found that 84% of his strains of streptococci, isolated from the blood of patients with rheumatoid arthritis, were in a narrow group. Typical strains taken from this group were agglutinated to a very high titre by the serum of patients with rheumatoid arthritis.

The agglutination test is conducted by growing the typical strain in a heart infusion medium for 24 to 48 hours and adding these freshly grown organisms to various dilutions (20 to 5120) of patient's serum. The dilutions are made with the heart infusion broth rather than the ordinary saline solution. After a primary incubation period of two hours at 56° C. in the water bath, the tubes are allowed to stand in the ice box overnight. Next morning, readings are made with the aid of a Kahn viewing box or other suitable device.

In most cases the agglutination is rather marked with the clumps large and not easily broken up. Some sera show a pro-agglutinoid

zone extending as high as 1-80. A few sera show agglutination beyond the 5120 tube although this dilution includes the majority of sera.

Table III shows a summary of agglutination tests.

TABLE III

AGGLUTINATION TESTS WITH STREPTOCOCCI

	Dilutions of Serum		
	0-80	160-320	640-5120
Rheumatoid Arthritis			
(Early)	24%	17%	59%
(Established)	26%	20%	54%
(Advanced)	30%	17%	53%
Osteoarthritis	83%	17%	0%
Arthralgia	48%	36%	16%

This simple procedure is apparently very reliable in detecting antibodies to more or less specific strains of streptococci, and may be compared to the Widal reaction in typhoid fever. It is uniformly present in a high percentage of the cases of rheumatoid arthritis, and never present to a high titre in uncomplicated osteoarthritis. Its interpretation, however, from a clinical point of view, is quite difficult.

Sedimentation Rate

This simple laboratory procedure is of great importance in the differentiation of rheumatoid and osteoarthritis.[12] A comparison between the two diseases is shown in Table IV.

TABLE IV

CORRECTED SEDIMENTATION RATE
MILLIMETERS PER HOUR

	0-10	10-20	20+
Rheumatoid Arthritis			
(Early)	23%	37%	40%
(Established)	14%	25%	61%
(Advanced)	8%	25%	67%
Osteoarthritis	85%	13%	2%

There are many methods for determining a sedimentation rate, but a method should be selected with a wide reading scale as comparative readings in rheumatoid arthritis are of considerable importance. Each specimen should be corrected to a constant cell volume and conducted at a constant temperature.

Rheumatoid arthritis always gives an accelerated rate, if the disease is active, and as progress is made in controlling the infection,

the rate decreases until it approaches normal. Osteoarthritis on the other hand shows a uniformly low or normal rate unless complicated by an infectious process.

Active Serum Complement Fixation

This more or less difficult procedure, first applied to arthritis by Burbank and Hadjopoulos,[13] is very useful in determining the reaction between antibodies and homologous bacteria. Theoretically, the thermolabile antibodies are not destroyed by inactivation and these combine with the homologous bacterial antigen, thus fixing the native human complement and causing inhibition of hemolysis and a positive result. Practically, it is subject to considerable error unless rigidly controlled. It is particularly useful in detecting the type of streptococcus or other organisms in the cases in which the agglutination reaction is negative or very low. Table V shows the results in a few cases.

TABLE V

ANTIGENS

	Streptococci						
	Beta	Alpha prime	Alpha	Gamma	Staphylococcus	Colon B.	Gonococcus
Rheumatoid							
Case O	+++	++	+++	+++	++	++	O
Case S	++++	+++	++++	++++	++	+++	O
Case Sh	O	++	+++	+	O	+	O
Case G	+	±	O	O	+	O	O
Case B	++++	++	++++	++	++++	++++	±
Osteoarth.							
Case R	O	O	O	O	O	O	O
Case Ra	+	O	O	O	O	O	O
Arthralgia							
Case A	+++	+	++	+++	++	+++	O
Case Au	O	O	O	O	+	O	O
Focal Inf.							
Case F	O	O	O	O	O	O	O
Case W	O	+	O	O	O	O	O
Case V	++	++	++	++	+++	+	++++
Case B	O	O	O	O	O	O	O

The object of all these cultures and tests is primarily for the purpose of selecting a suitable organism for vaccine preparation. The selection of a proper organism from a focus is difficult unless one is equipped to make a cataphoresis determination. Agglutination tests with autogenous freshly isolated strains of bacteria are not very practical as the organism usually shows marked auto-agglutination. Complement fixation is quite satisfactory, although the time

elapsing between isolating the organism, making the antigen and conducting the test is considerable. An organism isolated from the blood or joint fluid, stock strains agglutinated by the patient's serum and stock strains selected by complement fixation are the most reliable.

The vaccine is prepared by growing the organism in appropriate media until sedimentation occurs. The organisms are counted, washed with saline and killed chemically. Merthiolate has proven very satisfactory for this purpose in that the organism need not be washed of the chemical, as it is not toxic in the concentration used. The vaccine is made to a concentration of 1,000 million bacteria per c.c., and from this stock preparation various dilutions may be prepared for intravenous administration.

Summary

1. Streptococci have been isolated from the blood, joint fluid, lymph glands and foci of rheumatoid arthritis more frequently than any other organism.

2. A method that has been found satisfactory for the isolation of streptococci from the blood is presented.

3. Bacteriological, serological and electrophoresis studies suggest a streptococcus as the most common infectious agent in rheumatoid arthritis.

BIBLIOGRAPHY

1. Rosenow, E. C.: The Newer Bacteriology of Various Infections as Determined by Special Methods: Jour. Amer. Med. Assoc., 191, 13, 903.
2. Poston, M. A.: Gland Cultures in Infectious Arthritis, Jour. Amer. Assoc., 93, 692.
3. Clawson, B. J. and Wetherby, M.: An Experimental Basis for Intravenous Vaccine Therapy in Chronic Arthritis with a Summary of Results Obtained in Patients, Annals of Internal Medicine, 5, 12, June 1932, 1447-1461.
4. Gray, J. W. and Gowen, C. H.: The Role of the Streptococcus in Arthritis Deformans, Amer. Jour. Med. Sc., 1931, 182, 682.
5. Moon, V. C. and Edwards, S. H.: Results of Blood Cultures in Rheumatoid Arthritis, Jour. Infect. Dis., 1917, 21, 154.
6. Cecil, R. L., Nichols, E. E. and Stainsby, W. J.: The Bacteriology of the Blood and Joints in Chronic Infectious Arthritis, Arch. Int. Med., 1929, 11, 3, 571.
7. Klugh, G. T.: Streptococci from Blood Cultures in Arthritis, So. Med. Jour., 1931, 24, 706.

8. Gray, J. W. and Gowen, C. H.: A Bacteriologic Study of Chronic Infectious Arthritis, Jour. Med. Soc. of N. J., 28:38.

9. Gray, J. W., Fendrick, E. and Gowen, C. H.: Rheumatic Fever and Rheumatoid Arthritis, Texas State Jour. of Med., 28:203 (July) 1932; and Rheumatic Fever and Rheumatoid Arthritis from Laboratory Point of View, Texas State Jour. of Med., 28:327 (Sept., 1932).

10. Wood, W. L., Jensen, L. B., and Post, W. E.: Electrophoresis Studies in Cases of Focal Infection, Annals Int. Med., 8:734 (Dec.) 1934.

11. Nichols, E. E. and Stainsby, W. J.: Streptococcic Agglutinins in Chronic Infectious Arthritis, Jour. Clin. Invest., 1931, 10, 323.

12. Dawson, M. H., Sia, R. H. P. and Boots, R. H.: The Differential Diagnosis of Rheumatoid Arthritis and Osteoarthritis: The Sedimentation Reaction and Its Value, The Jour. of Lab. and Clin. Med., Aug., 1930, 15, 1065.

13. Burbank, R., and Hadjopoulos, L. G.: Serologic Significance of Streptococci in Arthritis and Allied Conditions, Jour. Amer. Med. Assoc., 84, 637, 1925.

CLOTTING INDICES: THEORY AND TECHNIC

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That blood has a clotting function has been known since ancient times, and the fact that certain substances, notably leech extract, deter clotting has been recognized for generations. It was not, however, until the work of Hewson, who showed clotting to be a problem primarily of the plasma, and of his successor, Schmidt, who in 1860 demonstrated that fibrinogen is converted to insoluble fibrin by the action of a ferment, thrombin, that the mechanism involved in blood clotting was actually studied.

Many theories have been set forth and many advances have been made toward explaining the complex physico-chemical reactions involved in the clotting of blood, and many factors have received exhaustive investigation.

The clotting of blood may be represented by the equations:

$$\text{Prothrombin} + \text{Platelets} \xrightarrow{Ca^{++}} \text{Thrombin}$$

$$\text{Thrombin} + \text{Fibrinogen} \longrightarrow \text{Fibrin (basis of clot)}$$

$$\uparrow \text{Antithrombin}$$

Arthus and Pages showed that calcium is essential for clotting and that it enters into the first step of the reaction. Calcium is only capable of acting as a catalyst when it occurs in the ion form.

On the addition of thrombin to a preparation of fibrinogen, fibrin precipitates. This reaction is the basis of blood clotting. It was shown by Howell that in mammalian blood this fibrin crystalizes in the form of fine fibrin needles. The insoluble fibrin contains less phospholipin than fibrinogen does.

The normal fibrin level varies considerably. The average value found by Gram, Foster and Whipple and McLester, and Foster and Whipple was 321 mgs. per 100 cubic centimeters of plasma; however, variations of from 15%-20% above or below this are to be considered normal. Howell gives normal values of from 0.22%-0.4%.

In addition to the factors in the equation which are working to carry the reaction to completion there is a factor, antithrombin, which is normally present in the blood in a small, but definite, amount which is acting in a reverse direction and tends to prevent clotting. The exact chemical nature of antithrombin is not known but it acts similarly to heparin or hirudin (leech extract). Applying the law of mass action to these equations it can be seen that any variation in the molecular concentration of the reacting substances would upset the equilibrium and that there would either be a greater or a less tendency to clot except as far as there would be a variation in the other factors to compensate for the shift. That the body is able to compensate for a sudden shift has been demonstrated by the fact that little or no change in the clotting reaction of the blood is evidenced after injections of even large doses of either thrombin or heparin (antithrombin).

In their work on the clotting index Drs. Bancroft and Stanley-Brown showed that the evaluation of the platelets or platelet lysis determinations shed no additional light on tendency to clot formation. These authors also report that "calcium and phosphorus determinations were also made on a small series (33 patients) but the variations from normal were so slight that these examinations were discontinued."[1]

These equations do not take into consideration elements arising from damage to vascular endothelium or factors arising from blood stasis which at times may enter into the reaction in the body; however, they include the principal factors which may be measured invitro.

Baumgarten in 1877 showed that stasis alone does not result in thrombosis. Miller and Rogers have proved that blood changes are prerequisite for thrombus formation.

With the above equations as a basis, the equation for the clotting index was derived:

$$\frac{\text{Prothrombin Index} \times \text{Fibrinogen}}{\text{Antithrombin Index}} = \text{Clotting Index}$$

The clotting index as given above was used by Drs. Bancroft and Stanley-Brown to show abnormal tendency to clot formation (thrombus or embolism) and has been used for this purpose at the Lancaster General Hospital. The technic, although not original, has been modified sufficiently to justify being given in detail.

Technic:

Take 9 ml. of blood from the vein, special care being taken, first, to avoid any clot formation by rinsing the syringe with 1% sodium oxalate, and second, to avoid stasis as far as possible. Mix the blood at once with 1 ml. of 1% sodium oxalate solution and centrifuge at low speed for 3 minutes. Long centrifuging and centrifuging at high speed alter the clotting time of the plasma.[8] Remove the plasma and use for the following tests:

Prothrombin Index. Place 0.1 ml. of plasma in each of 3 test tubes (6.8 x 1.3 cm.) with flat bottoms and to these add 0.1, 0.2 and 0.3 ml. of 0.5% calcium chloride respectively. The shortest clotting time in the series is the prothrombin time. Determine the prothrombin time of plasma from a normal person at the same time as the test. This is known as the control time.

$$\text{Prothrombin Index} = \frac{\text{Time of clotting of control plasma}}{\text{Time of clotting of patient's plasma}}$$

Fibrin:

To 1 ml. of plasma add 28 mls. of 0.8% sodium chloride solution and 1 ml. of 2.5% calcium chloride. Mix and allow to stand for 20 minutes. Remove the fibrin web that has formed by twisting around a glass stirring rod; expell as much solution from the clot as possible by pressing against the side of the test tube. Blot the fibrin lump dry with filter paper. Care must be taken to remove any adhering solution. Place the small lump of fibrin in a 15 ml. centrifuge tube and add 4 ml. of 0.25 N sodium hydroxide. Place in a boiling water bath until the fibrin lump is completely dissolved. Centrifuge. Pour supernatant fluid into a 25 ml. volumetric flask and fill to approximately 20 ml. with distilled water. Add 1 ml. of 1 N sulphuric acid and 0.5 ml. phenol reagent. Add 1 ml. 2.5 N sodium hydroxide and dilute to mark with distilled water. Mix thoroughly. Let stand 1 hour. The standard is prepared at the same time as follows: place 1 ml. of standard tyrosine solution (1 ml. equals 0.2 mgs.) in a 25 ml. volumetric flask. Add 0.5 ml. of phenol reagent. Dilute to approximately 20 ml. Add 1 ml. of 2.5 N sodium hydroxide. Dilute to volume. Let stand 1 hour before comparing in the colorimeter. Set the unknown at 16.4, then 0.02 times the reading of the standard divided by 0.9 equals the percentage of fibrin.

Antithrombin Test:

Heat about 1 ml. of the control plasma, and the plasma to be tested, at 60° C. for 10 minutes. Prothrombin is destroyed and fibrin is precipitated. Centrifuge for 5 minutes. Use supernatant fluid in the following test: Prepare normal plasma the same way as the plasma to be tested and place 0.1 ml. in each of 5 tubes (the third and fourth tubes serve as the control), the same size as those used for the prothrombin index. To the first tube add 0.06 ml. heated test plasma and 0.04 ml. 0.9% sodium chloride. To the second tube add 0.1 ml. heated test plasma. To the third tube add 0.06 ml. heated control plasma and 0.04 ml. 0.9% sodium chloride. To the fourth tube add 0.1 ml. heated control plasma. To the fifth tube, which serves as a check on the prothrombin time of the normal plasma, add 0.1 ml. 0.9% sodium chloride. Shake all tubes and allow to stand 20 minutes at 37° C. Several tests may be run simultaneously with one control test. Add 0.2 ml. 0.5% calcium chloride to each tube and note clotting time.

$$\text{Antithrombin Index} = \frac{\text{Time of clotting of test}}{\text{Time of clotting of control}}$$

$$\text{Clotting Index} = \frac{\text{Prothrombin index} \times \text{fibrin}}{\text{Antithrombin index}}$$

*Comment: We realize that a certain percentage of error is introduced by carrying out the prothrombin test and antithrombin test at room temperature. We feel, however, that this error is minimized by performing the control test and the patient's test at the same temperature and time. The blood for the control test is either taken from a known normal person in the laboratory or a patient in the clinic who is known to have no clotting disturbance or bacterial infection.

The following chart gives a summary of the clotting indices 1933-35:

CHART I

Operation or type of case	No. of cases	No evidence of clot		Definite thrombus			High clotting Index No definite clot			
		Normal No. of cases	C. I. Aver. C. I.	No. of cases	C. I. before treatment	C. I. after treatment	Normal recovery	Stormy recovery	C. I. before treatment	C. I. after treatment
Appendectomy	138	116	0.54	6	0.91	0.63	7	9	0.85	0.58
Gynecological	230	150	0 54	19	0.95	0.53	37	24	0.81	0.52
Cholecystectomy	105	59	0.52	6	0.93	0.62	20	20	0.85	0.56
Herniorrhaphy	60	44	0.49	1	0.83		10	5	0.84	0.40
Obstetrical	92	67	0.51	8	0.95	0.51	12	5	0.84	0.58
Prostatectomy	44	27	0.55	2	0.85	0.60	11	4	0.81	0.51
Gastro-Intest.	35	20	0.53	0			11	4	0.89	0.54
Thyroidectomy	25	21	0.54	1		0.65	2	0	0.80	0.58
Fracture	30	25	0.50	1	1.30	0.53	4	1	0.93	0.54
Coronary Thrombosis	15			15	0.47					
Infection	15	1	0.42	1	(B. Welchii) 0.79			13	0.90	
Miscellaneous	224									

In the group of cholecystectomies, one case which at autopsy was found to have a pulmonary embolism had a high clotting index two days post-operatively. She received no sodium thiosulphate but received large amounts of intravenous glucose and saline. Despite this treatment on the fifth day post-operative a pulmonary embolism developed.

In the group of obstetrical cases one patient who had a caesarian section and developed a thrombophlebitis, at no time up to the date of its development had a high clotting index. Anemia was present, red blood count 2,960,000, hemoglobin 52%. Whether this was a factor in causing the normal clotting index is a matter of conjecture.

The finding of a normal clotting index in the 15 cases of coronary thrombosis was interesting, as the primary change and factor in causing the thrombus formation in these cases, is, according to

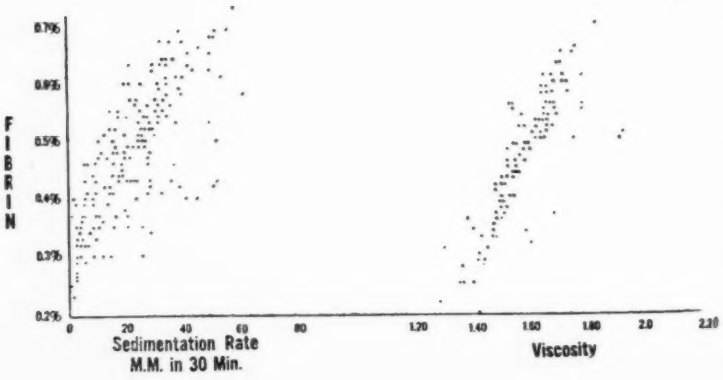
one of the latest theories, in the blood vessel walls and not in the chemical constituents of the blood.

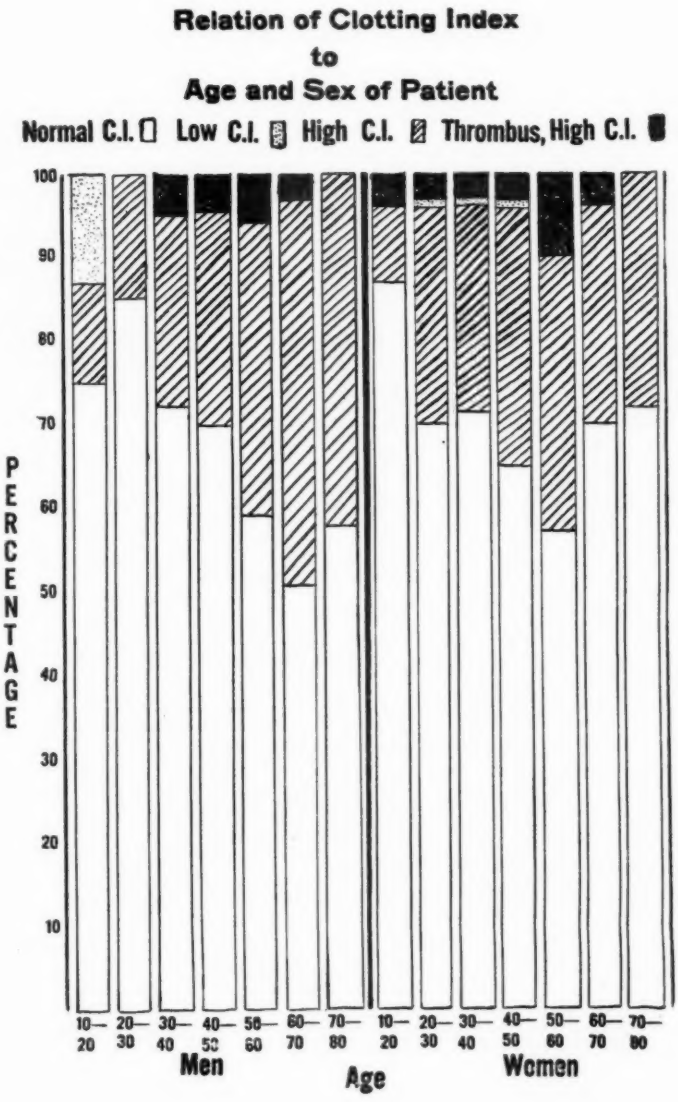
In the group of infections the fibrins were very high, several being 0.9 or above. Several bacteria, especially staphylococci, seem to cause the formation of abnormally large amounts of fibrin, while others such as hemolytic streptococci seem to have a definite fibrinolytic action.

On a series of 200 tests in addition to the clotting index the sedimentation rate was determined. Linton[8] has used the sedimentation rate as a means of indicating post-operative tendency to bleed. There was found to be a certain degree of parallelism between the sedimentation rate and the fibrin content. However, in some cases of severe infection the sedimentation rate was disproportionately high. This may have been due in some instances to the fibrinolytic action of the bacteria causing the infection. No relationship between the sedimentation rate and the clotting index was noted. From this group of tests we feel that the clotting index gives information which cannot be derived from the sedimentation rate.

On a series of 200 tests the viscosity of the blood plasma used for the clotting index was determined. The fibrin and viscosity were found to be directly proportional. However, in cases of diabetes in which the sugar level was above normal limits the viscosity was disproportionately high. Fig. 1.

Relation of Fibrin to Sedimentation Rate and Plasma Viscosity




Relation of Clotting Index
to
Age and Sex of Patient
Normal C.I.
Low C.I.
High C.I.
Thrombus, High C.I.
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
10— 20
20— 30
30— 40
40— 50
50— 60
60— 70
70— 80
Men
Age
Women

The greatest percentage of thromboses and emboli were found in women between the ages of 50 to 60 and in men between the ages of 50 to 60. However, the greatest percentage of abnormal clotting function was found in men between the ages of 60 to 70. Fig. 2.

Conclusions:

The clotting index is raised in most cases of thrombosis and embolism. It is, however, normal in cases of coronary thrombosis.

The blood fibrin is increased in cases of infection caused by certain bacteria as staphylococci and may be decreased in cases of infection caused by fibrinolytic bacteria as hemolytic streptococci. There is a certain degree of parallelism between the sedimentation rate and the fibrin content of the blood, however, the clotting index gives information not supplied by the sedimentation rate.

The fibrin content and the viscosity of the plasma are in most instances in direct proportion.

It is believed that further study and work along this line will extend the clinical applications of the clotting index.

(I wish to acknowledge the interest and co-operation of Dr. Louisa E. Keasbey, Pathologist, Lancaster General Hospital, upon whose suggestion the work on the Clotting Index was undertaken at the Lancaster General Hospital and the help of Miss Anna Mary H. Falck, L.T., who gave much aid in the compiling of statistics.)

BIBLIOGRAPHY

1. Bancroft, F. W. and Stanley-Brown, M., Postoperative Thrombosis Thrombophlebitis and Embolism. Surg., Gynec. and Obst. 54: 898-906, June, 1932. Evaluation of Blood Clotting Factors in Surgical Diseases. Annals of Surgery xc P. 161, 1929.
2. Ersner, M. S., Myers, D. and Ersner, W., Clinical and Experimental Study of the Action of Saliva on Blood Coagulation and Wound Healing in Surgery of the Oral Cavity and Throat. Ann. Otal., Rhin. and Laryng. 43: 114-145, Mar., 1934.
3. Foster, D. P., A Clinical Study of Blood Fibrin. Arch. Int. Med., Vol. 34, p. 301, 1924.
4. Gilligan, D. R. and Ernstene, A. C., The Relationship Between the Erythrocyte Sedimentation Rate and the Fibrinogen Content of the Plasma. Am. J. Med. Sciences, Vol. 187, No. 4, p. 552, Apr., 1934.
5. Kugelmass, I. N., Determination of Blood Clotting Function. New York State J. Med. 31:759-76, June 15, 1931. Mechanism and Management of Haemorrhagic Disturbances in Infancy and Childhood. New York State J. Med. 33: 434-447, Apr. 1, 1933.
6. Kugelmass, I. N. and Samuel, E. L., Avitaminosis and Blood Clotting Function. Am. J. Diseases of Child. Vol. 43, p. 52.

7. Lewiskohn, R., Haematologic Studies as a Basis for Determining Risk of Post-Operative Haemorrhage in Jaundice Patients. Ann. Surg. 44:80-87, July, 1931.
8. Linton, R. R., The Sedimentation Rate of Blood as an Index of the Haemorrhagic Tendency Obstructive Jaundice. Ann. Surg., Vol. xci, p. 694, 1930.
9. McLester, J. S., Diagnostic Value of Blood Fibrin Determination with Special Reference to Diseases of the Liver. J. A. M. A., xxix,, p. 17, 1922.
10. Miller, R. H. and Rogers, H., Postoperative Embolism and Phlebitis. J. A. M. A., Vol. 93, No. 19, p. 145.
11. Nygaard, K. K., Coagubility of Blood Plasma. Staff Meetings of the Mayo Clinic. P. 151, Mar. 7, 1934.
12. Ravdin, Riegel and Morrison, Coagulation of the Blood. Ann. of Surg., Vol. xci, p. 801, 1930.
13. Rosenthal, S. R., General Review—Thrombus and Fatal Pulmonary Embolism. Archives of Pathology, Vol. 11, 14 No. 2, pp. 121-124.
14. Sanford, H. N., Gasteyer, T. H., Wyat, L., The Substances Involved in the Coagulation of the Blood of the New Born. Am. J. Diseases Ch., Vol. 43, No. 1, p. 57, Jan., 1932; No. 3, p. 566, Mar., 1932.
15. Review from Archiv. fur klinische chirurgie, p. 252, Sept. 20, 1933. J. A. M. A., 11, 25, 1934, p. 1764.
16. Wu, H. and Ling, S. M., Colorimetric Determination of Proteins in Plasma, Cerebrospinal Fluid and Urine. Chinese J. Physiol., 1927, 1, 161.
17. Wu, H., A New Colorimetric Method for the Determination of Plasma Proteins. J. Biol. Chem., 51, 33, 1922.
18. Howell, Wm. H., Text Book of Physiology.
19. Macleod, J. J. R., Physiology and Biochemistry in Modern Medicine.

PATHOLOGICAL TECHNIC

By MARTHA R. MOORE, L.T.

Jacksonville, Fla.

The article on "Aspiration Biopsies," by Martin (1) describes the technic in detail and reports that over 1,400 positive histological diagnoses of cancer have been made by this method. The fresh tissue fragment on the glass slide is smeared by firm, flat pressure by another slide, drawn once across. The smeared slide is fixed by heating gently over a gas flame until warm and dry and is then prepared according to the following immediate method:

1. Alcohol (95%) — one minute
2. Water — one minute
3. Hematoxylin — one minute
4. Water — one minute
5. Check by microscopic examination of the cellular stain and if sufficient, proceed, or if insufficient, return to hematoxylin for deeper staining.
6. Eosin — one minute (Check stain microscopically)
7. Alcohol (95%) — one-half minute
8. Carbol-xylol — one-half minute
9. Xylol — one-half minute
10. Mount with Canada Balsam and cover glass.

The remainder of the specimen, consisting of collected tissue fragments or blood clot containing them, is treated as any small biopsy.

The article by Reich, "A Modified Technic for Sternal Puncture and Its Value in Hematologic Diagnosis" (2), discusses preparing and staining bone marrow in diagnosis of anemias. This method as outlined is simple and easily adapted to routine hospital use:

1. Take 10 c.c. of aspirated fluid in 15 c.c. centrifuge tube mixed with 2 c.c. of a 1.4% Sodium Oxalate solution; that is, an "isotonic anticoagulant which does not change the volume of the cells."
2. Centrifuge about five minutes at moderate speed.
3. Decant supernatant plasma.
4. With a pipette transfer the buffy coat to a tube made by sealing off one end of a short piece of glass tubing with diameter slightly larger than the pipette.
5. Centrifuge again for five minutes.
6. Remove plasma.
7. Make smears from the buffy coat on slides and stain as follows:
   Jenner stain — 3 minutes
   Distilled water — 3 minutes
   Decant
   Flood with dilute Giemsa 30 minutes, 1 drop to 1 c.c.
   Wash and dry.
8. Count 1000 cells for differential.

Lubarsch's Method (3) for preparing paraffin sections and the one by MacCarty and Wellbrook (4) are both well known. "A Modified Routine Method for Rapid Paraffin Sections" is characterized by simplicity of procedure, economy of materials, rapidity of dehydration and constancy of results with the addition of heat and without xylol, toluol, chloroform, or oil. The tissue blocks should be cut thin, 1-3 mm. thick and put into small bottles with numbered tags.

I. Fixation
   A. Formalin 10% for ten minutes in water bath at 56° C.
   B. Pour off fluids each time instead of transferring tissues.

II. Dehydration
   A. Alcohol (95%) for ten minutes in water bath at 56° C. (A properly denatured alcohol is satisfactory. Use solutions only once.)
   B. Absolute alcohol for ten minutes in water bath at 56° C.
   C. Acetone for ten minutes in water bath at 56° C. (Waste acetone may be pooled for cleaning balsam or oil from slides.)
   D. Acetone for ten minutes in water bath at 56° C. (Blot tissues before putting them into paraffin.)

III. Infiltration
   A. Paraffin (54° C.) for one hour. If the laboratory does not have a paraffin oven substitute an electric globe and a common green metal shade. As long as some of the paraffin remains solidified around the edges of the containers, the tissues will not be overheated.

IV. Embedding
   A. Use paraffin containing 20% beeswax which does not necessarily require rapid hardening, but if time is a factor place embedding boxes on ice. These boxes are composed of copper plates and lead "L's". By arranging these "L's" in various positions different tissues can be embedded in the least amount of paraffin and in different shapes, square or rectangular, and the blocks will not have to be trimmed.

V. Mounting
   A. Mount paraffin blocks on blocks of wood. With the microburner in the right hand, hold the tissue block in the left hand; so that, the surface to be sectioned will be downward. On this surface the number of the specimen may be written. Flame the upward surface of the tissue block until it is smooth and soft. Heat the wooden block until the paraffin oozes from it. Quickly press the two warm surfaces of the paraffin block and the wooden block together to make them stick. Flame around the edges of the block. (New wooden blocks should first be soaked in hot paraffin).

VI. Sectioning

A. Chill the tissue blocks with ice.

B. Write number on envelope in which the paraffin block is to be filed.

C. Place block in microtome parallel with a sharp knife which can be kept in good condition in the laboratory by using a Schmid Microtome Knife Sharpener hand model. A slab of plate glass, 18 x 13½ x ½ inches, under which a damp cloth is stretched to prevent the plate from slipping is used with an abrasive such as carborundum powder for grinding the nicks out of the knife, and a polisher such as Eureka Diamantine. The abrasive and polishing powder are mixed with water and glycerine. Handle the knife carefully and keep it always oiled while not in use.

D. Cut sections 6 micra thick. (Much difficulty may be avoided by keeping tissue block, knife, forceps, and teasing needle cold).

E. Remove the ribboned sections from the knife by taking a small pair of forceps in the right hand and picking up the first section of the ribbon while holding the teasing needle in the left hand and using the index finger to free the section from the bottom edge of the knife. Avoid letting the instruments and ice come into contact with the edge of the knife if you want an edge without nicks.

F. Carry the ribbon to the right of the microtome and lower the free end into the cold tap water. (Use the teasing needle and the forceps with an opening and closing effect of the latter to straighten out small wrinkles).

G. Write number on glass slide and with the finger smear drop of egg albumen making a very thin film.

H. Take the slide in the left hand, the teasing needle in the right hand. Place the slide in water beneath the section and tease section into position.

I. Immediately transfer the slide to warm water about 58° C. to flatten out the section.

J. Drain slide thirty minutes or longer before gently heating it at intervals over a gas flame and letting it cool each time. (The section is fixed to the slide when it loses its opacity and takes on a translucent appearance).

VII. Stain as usual.

1. Xylol, 2 minutes.
2. Xylol, 2 minutes.
3. Absolute alcohol, 2 minutes.
4. Alcohol (95%), 2 minutes.
5. Wash in water.
6. Hematoxylin, 4-10 minutes. Check microscopically. (Preferably Gruebler's products.)
7. Wash in water.
8. Decolorize, if necessary, in 70% alcohol containing 1% HCl. (When the rose color appears quickly wash in water until the blue color returns.)

9. Eosin, one minute.
10. Alcohol (95%), one-half minute.
11. Absolute alcohol, one-half minute.
12. Creosote, one-half minute.
13. Drain, two minutes.
14. Mount with Canada Balsam and cover glass.

The following simple method is suggested for the examination of the body fluids for cytologic study. These are precipitated with alcohol; centrifuged ½ to 1 hour in large conical tubes; washed in saline several times by centrifuging and pouring off the supernatant fluid. Then the sediment is covered with 10% formalin and a teasing needle is run along the side of the tube in several places to aid the penetration of the fixative. This tube is placed in a water bath at 56° C. for thirty minutes. After the fluid is removed the clot is carefully taken out. A longitudinal section is cut and put into a bottle and is ready for the dehydration as previously outlined.

Summary

The chief purpose of this paper is: first, to review the explanations as given on aspiration biopsies and biopsies of bone marrow in anemias as special methods; secondly, to present a modified routine method with rapid dehydration for paraffin sections and also helpful suggestions in the different steps of this pathological technic; and thirdly, to submit the procedure that I am using for hardening and dehydrating fluids for cytologic study.

BIBLIOGRAPHY

1. Martin, Hayes E. and Ellis, Edward B.: Aspiration Biopsy, Surgery, Gynecology and Obstetrics, 59:578-589 (Oct.) 1934.
2. Reich, Carl: A Modified Technic for Sternal Puncture and Its Value in Hematologic Diagnosis, The Journal of Laboratory and Clinical Medicine, 20:286-288 (Dec.) 1934.
3. Stitt, E. R.: Method of Lubarsch, Practical Bacteriology, Blood Work, and Animal Parasitology, p. 622.
4. MacCarty, Wm. C. and Wellbrook, W. L. A.: Preparing and Staining of Paraffin Sections, Kolmer and Boerner's Approved Laboratory Technic, pp. 614-615.

EDITORIALS

THE IDEAL MEDICAL TECHNOLOGIST

Medical technology as we know it today had its beginnings in 1881. In that year, Frau Koch prepared some gelatin in her kitchen and on that gelatin her husband first isolated bacteria in pure culture. If Koch had been a bachelor with a lazy and disinterested servant girl in his kitchen, he might never have formulated his postulates and the science of epidemiology might have had to wait for another sire. Frau Koch is the prototype of the modern laboratory technologist. Her work may not be as important in its implications as that of the doctor but it is just as essential that it be done carefully and well. The technologist and the physician form a mutually dependent team which functions to better advantage than if either of them worked alone. The busy practitioner of medicine finds no time to perform highly technical procedures which are daily becoming more numerous and involved. The technologist has neither the inclination, training nor experience to interpret the results of these tests.

Recently there has arisen a rather heated and unnecessary controversy as to just what kind of training and education the technologist should have. Quite obviously, she must have enough knowledge of the interpretation and application of the tests she runs, to make their performance a rational rather than a mechanical procedure. Otherwise she will degenerate into a self-centered robot lacking in scientific honesty, ambition and energy. She will often perform "sink tests" when nobody is looking, quite regardless of the reputation of the laboratory and her chief, and quite heedless of the lives of her patients. Just as obviously on the other hand, she must realize fully the limitations of such knowledge as she possesses, or she might assume the role of diagnostician and impart medical advice like any other well-meaning quack, with equally disastrous results for laboratory and patient.

I have been a follower in the footsteps of Frau Koch for thirteen

This is the first of a series of editorials on this subject. The next issue will contain an editorial by Dr. Maurice Fishbein, editor of the Journal of the A. M. A. Future contributors will include Dr. Malcolm T. MacEachern, Acting Director of the American College of Surgeons, Dr. John Lanford, Department of Pathology, Tulane University, Louisiana, and Dr. James S. McLester, president of the American Medical Association for 1935-36.—Ed.

years and naturally I have formed some idea as to just what sort of person makes the best medical technologist and just what sort of personality, preliminary education and experience she should have and what the curriculum of the training school should include. During these thirteen years it has been my good fortune to act as guide and mentor to more than fifty student technologists and to know intimately or work daily with half a hundred more. None of us have been that paragon, the ideal technologist, but if from these hundred people I could prune the faults and nourish the virtues, I believe I could construct an almost perfect one. My formula for synthesizing her would be somewhat as follows:

In the first place, make a very careful selection of the applicants who enter the training school. Just as nature in fashioning a new individual, starts with certain basic factors which we call genes, so the maker of technologists must start with people. And if your list of applicants for the training school is long enough you can further imitate nature and select the genes which will be most adaptable and desirable. I should select women rather than men, not because I think women make better technologists than men, but because the organization of the training school usually delegates the supervision of the younger technologist to women, and a young lady would find herself in a more congenial atmosphere than a young man similarly placed. No man likes to know that a woman is the boss. Then pick out a young girl with a healthy body, an alert mind and a good disposition. The early twenties is a suitable age in which to start her training. By then she will be old enough to have some sense of proportion and young enough to be adaptable. See that she has a good supply of common sense and a sense of humor. These two characteristics can never be acquired but are absolutely essential. Next in importance come good breeding, good looks and good manners. I do not mean that she must be of exceptional beauty but she must have a neat personal appearance and pay some attention to dress and the care of person. The good manners can be taught, but the process is a tempestuous one and its accomplishment may disrupt the nicely balanced machinery of co-operation between the laboratory, the doctors and the nursing staff. Finally select a girl with a good scientific training from a university. Lack of such a background may be partially remedied by insisting that she take scholastic training at a local college during her period of training or by giving her didactic instruction in chemistry, physics and biology. But the facilities in most hospitals for teaching basic sciences are inadequate and the laboratory staff is usually so overworked that they can find scant time to hear the lessons of the students or to see that they do their "home work" properly.

Then take this fledgling under your wing and watch and instruct her by precept and example as tenderly as if she were your own child. For six months, keep her rather closely confined to the laboratory. During this time have her learn thoroughly the uninteresting routine of urinalysis, of blood counting and simple determinations in biochemistry. This period is a trial by ordeal and discourages all the students except the ones who really love the laboratory. Do not make it too drab. Let the neophyte observe the more complicated and exciting procedures. Also introduce her to the great American public as seen through the doors of the laboratory. Let her meet the grateful as well as the ungrateful patient, let her see the mother who thinks we are inhuman because we "stick" her baby in the jugular vein as well as the mother who bakes us a cake because we found hookworm or malaria and her child is getting fat and healthy again since the doctor put him on the new medicine. And let her see the visiting and resident staff in all their impatient, generous, unreasonable and temperamental moods. But be sure she can discern that their faults and the adverse criticism they make of her work are motivated by the high ideal of their desire to serve their patients better and to heal disease. Then she will begin to realize her responsibility in doing her own work carefully so that she can help the doctor make a correct diagnosis, institute rational therapy, cure the patient and get another vacant bed in the hospital for some waiting patient.

When the technologist has learned to respect the staff and has become familiar with the routine of the laboratory, when she can make a dependable urinalysis and a correct blood count, when she can get into a vein without subjecting the nerves of her sister technologists and her patients to undue strain, she is ready to begin her second six-months' training period. She now goes into the wards with the older technologists. She helps collect blood counts, Wassermanns and chemistries, observes the metabolisms and E.K.G.'s, follows the medical staff in ward rounds and occasionally attends an autopsy and a clinical-pathological conference. Encourage her to fraternize with her fellow workers, the nurses, and have her attend some of their lectures, especially the ones in microbiology and pathology. During this period the enormity of being careless with her work will be apparent to her. She will see that she cannot afford to make a mistake because the results of it may mean the life or death of a human being.

The final six months of her training should be spent in co-ordinating all her knowledge and in running all the laboratory procedures by herself. At this time, she might be compared to a six months human fetus. She is perfectly formed but unable to function, that

is not viable, if removed from the sympathetic atmosphere of the teaching laboratory to a place of her own in the grown-up world. During this period it is most important that her superiors be people who possess the best of medical and laboratory tradition and training as well as a thorough understanding of human relationships. And it is at this time that she has most need of her common sense and judgment. She must begin to learn when to consult authority and when to act on her own initiative. She knows how to do all these tests and is highly skilled and facile in the science, but she has no appreciation of the art, of medical technology. She must start acquiring it during this final six months.

At the end of this time the full-term baby technologist will be born and her laboratory parents will think she is ideal. She will be able to do her work well in any laboratory anywhere. She will be thoroughly familiar with the usual ways of doing the usual procedures but she will be inventive enough to modify and alter her technic, and original enough to think up better ways of doing things. She will eagerly spend long hours in monotonous routine, but she will find time to read extensively and introduce new procedures into her laboratory whenever they are asked for, or when she herself thinks they will improve the laboratory service. She will have sufficient foresight to eat her meals regularly, to sleep all she can, and to relax and enjoy herself whenever she has opportunity so that her health will not become impaired nor her mental outlook dull. She will be a pleasant, likable person and will look neat and presentable in her clothes. She will co-operate cheerfully with the doctors and nurses and will know how to bear criticism with dignity and praise with humility.

Even should she find herself a job in a laboratory which is an undesirable one, this will not impede her further progress and development, for she will either find herself a better place to work, or she will alter her laboratory and remodel it into the pattern of the teaching laboratory which gave her nurture and birth.

—S. Mc.

THE FOURTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL LABORATORY TECHNICIANS

On May 11, 12 and 13 the fourth annual convention was held at the Elms Hotel, Excelsior Springs, Mo. Those who attended found awaiting them a very hospitable meeting place, surrounded by a most congenial atmosphere.

Although not attended by as many members as the convention of last year, a far better representation by states, was present. Considering the fact that the greatest number of members reside in the eastern section we are safe in saying the fewer numbers present at this meeting represented a much larger territory geographically, and proportionately a more widespread and greater interest than yet shown.

Due to a more extensive program of speakers and several business sessions, the plans for social activities were greatly curtailed. It is hoped that time may permit some organized social programs, aside from the annual banquet, for the next convention.

The scientific exhibits were of much interest and value. An actual demonstration does much toward impressing the merit of any procedure. Displays presented represented the five sections of the country.

The papers read reached into the storehouses of knowledge for new concepts of bacteriology, mycology, parisitology, haemotology, serology, histology, biochemical analysis and problems, varied in nature, relative to the clinical laboratory and the personnel. Space does not permit a review of the scientific data given but many of the papers will be printed in the near future.

A brief resumé of the business sessions of the convention may be found under "News and Announcements." Special attention should be called to the fact that the official title of the organization has been changed to "The American Society of Medical Technologists." This title was adopted in as much as the classification as granted to all registrants by the Board of Registry will be "Medical Technogolists." It was also announced by the Registry in joint session with the Executive Committee that it shall be known as the "Registry of Medical Technologists" of the A. S. C. P. Notice pertaining to same is printed elsewhere in this issue of the Journal.

NEWS AND ANNOUNCEMENTS

NATIONAL

PROCEEDINGS OF THE FOURTH ANNUAL CONVENTION

The following will summarize somewhat briefly, the minutes of the meeting held May 11, 12 and 13 at Excelsior Springs, Mo.:

Called to order by the President, Robert C. Jenkins, followed with invocation by Rev. W. L. Brown. In the absence of Miss Madie Murphy, secretary, Miss Luella Gifford was appointed acting secretary for the convention. Minutes of previous meeting were read and approved.

The President made the following committee appointments:

Nominating Committee: Vern Flannery, representing northeast, chairman; Bernice Elliott, representing northwest; Hermaine Tate, representing southwest; Sarah McCarty, representing southeast; Christine Seguin, representing central.

Resolutions Committee: Phyllis Stanley, chairman; Pearl Moorman, Sister Alcuin, Marian Baker.

Reports of the Executive and Research Committees, Advisory Board, Treasurer, Publication and Membership, were read and adopted.

Motion adopted that Article I of the Constitution shall read: **"This organization shall be known as The American Society of Medical Technologists."** Also that the name of the official publication, "The American Journal of Medical Technology" be patented and that its contents be copyrighted.

Motion adopted that a copy of the constitution and by-laws be sent to each member as soon as possible after the first of the fiscal year. A few minor changes were made in the constitution which will be apparent when the new copy is received.

Balloting on names brought forward by the Nominating Committee resulted in the election of the following:

Frieda Claussen—President.
Sister Alcuin—Vice-President.
Ann Snow—Secretary.

Executive Committee:
Vern Flannery and Lawrence Ray.
Vivian Herrick—unexpired term.

Advisory Board: Lona Jacobson.

President Elect: Sister M. Joan of Arc Wilson.

Meeting adjourned.

AWARDS—FOURTH ANNUAL CONVENTION

1. First Award (Silver Medal)—Phyllis Stanley, M.S., M.T., Newark, N. J., "The Preparation of Transudates for Histological Study," combined with the excellent exhibit, personally prepared to demonstrate the results of her technic, and the presentation of her own museum preparations and mounted photographs.

2. Second Award (Bronze Medals)—Annette Callan, M.T., Philadelphia, Pa., "Plain Facts About Blood Cultures." The Texas Society of Medical Technologists, Anna Lou Smith, L.T., Secretary, Fort Worth, Tex. "Model Laboratory," a group exhibit.

3. Honorable Mention (Certificates)—Anna Snow, L.T., Little Rock, Ark., "Laboratory Recognition of Monilia." Bernice Elliott, B.S., M.T., Omaha, Neb., "Folin Berglund Method for the Quantitative Determination of Glucose in Urine." Hermine Tate, L.T., Jackson, La., "Comparison of the Kolmer Wassermann, Kahn and Johns Precipitation Tests."

POSTPONEMENT NOTICE—The First International Conference on Fever Therapy, originally scheduled for the end of September, 1936, has been postponed because of numerous requests, to permit more time for the preparation of material. The new dates set for this Conference are March 30th to April 2nd, 1937. The sessions will be held at the College of Physicians and Surgeons, Columbia University, New York City.

We wish to call your attention to the offer of free literature by Fisher Scientific Co. pertaining to their new direct-reading Photometric Colorimeter, also the literature as supplied by the H. Reeve & Co., Inc., 7-11 Spruce St., New York, pertaining to the selection of filter papers. Please mention Journal when writing advertisers.

OFFICERS A. S. M. T.
1936-37

FRIEDA CLAUSSEN, M.T.
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Treasurer

EXHIBITS

Annual Convention

MAY
1936

STATES

Colorado

The Colorado Society of Clinical Laboratory Technicians was organized November 19, 1930, with 13 charter members. Membership has gradually increased until there are now 50 enrolled.

Regular programs have consisted of talks by medical authorities in special fields, reports on special assignments to members of the society, and demonstrations of new and established technical procedures.

On Saturday, March 28, 1936, the first Annual Convention was held by the Society at the University of Colorado School of Medicine in Denver. The program began at 10:30 a. m., with registration of the members and an address of welcome by the president. An inspection of 13 scientific exhibits followed.

There were also exhibits by local commercial laboratory equipment houses and one exhibit of several pieces of antique laboratory apparatus presented by the medical school.

At noon a luncheon was held for delegates in the dining room of the Medical School.

The afternoon of the convention was given over to the following program:

1. "Recent Work in Hemoglobin Studies" by Dr. E. R. Mugrage, Director of Clinical Laboratories, University of Colorado Medical School.
2. "A Study of Living Blood Cells by Means of Motion Pictures" by Dr. Hugh Kingery of the University of Colorado Medical School.
3. "Differential Diagnosis of Mononuclear Blood Cells" by Lavina White, Pueblo Clinic, Pueblo, Colorado.
4. "Nutrition and Toxology" by Dr. B. F. Poe, Department of Chemistry, University of Colorado, Boulder, Colorado.

On May 5, 1936, the following officers were elected to serve during the year 1936-1937:

President—Irma A. Bair.
Vice-President—Loretta Hamilton.
Recording Secretary—Jerry McNamara.
Corresponding Secretary—Allyne Lawless.
Treasurer—Mary Dilly.

Note: Members and officers of the Colorado Society of Clinical Laboratory Technicians can be reached through the National Registry, Room 234, Metropolitan Building, Denver, Colorado.

Ohio

The Third Annual Convention of the American Society of Clinical Laboratory Technicians of Ohio, held in Columbus, Ohio, April 15th, at the Deshler-Wallick Hotel.

The Business Session was opened at 11:00 A. M. Alice Finnin, President, presiding with twenty-six (26) Technicians and one (1) Pathologist present.

The minutes were read and approved.

The report of the President was read by Alice Finnin.

The report of the Secretary was read by Martha Andes.

The report of the Treasurer was read by Ruth Koons.

The reports were accepted and placed on file.

In the absence of the members of the Executive Committee a motion was made and seconded to make necessary changes and amendments to the Constitution.

The business session adjourned at 11:35 A. M.

At 2:00 P. M. Wednesday, April 15th, the Technicians attended the Joint Meeting of the Ohio Hospital Association; George Wilson, Superintendent, Toledo Hospital, presiding.

A round table discussion entitled "Professional Problems" was conducted by R. C. Buerki, M.D., President of the American Hospital Association.

Wednesday afternoon at 3:30, the meeting opened with 43 Technicians and 2 Pathologists present, Alice Finnin presiding.

Dr. M. F. Steele, President of the Ohio Hospital Association, gave an address of welcome.

Words of encouragement and good-will were given by Dr. R. C. Buerki, President of the American Hospital Association.

Dr. C. L. Spohr, Professor of Clinical Pathology, Ohio State University, Columbus, presented "Oxydase Reaction for the Identification of Gonococcal Colonies."

Dr. R. S. Fidler, M.D., White Cross Hospital, Columbus, presented "Glucose Tolerance Tests in Relation to Pituitary and Endometrial Dysfunction."

Miss Rose Ketteringham, M.T., Toledo Hospital, Toledo, gave a paper and presented slides and literature on "Malaria from the Viewpoint of the Ohio Technician."

The meeting adjourned at 5:30 P. M.

An informal dinner was held at 6:30 P. M. with Dr. F. C. Potter, Pathologist, Peoples Hospital, Akron, Toastmaster.

BOOK REVIEW

THE SPECIFICITY OF SEROLOGICAL REACTIONS. By Karl Landsteiner, M.D., The Rockefeller Institute for Medical Research, New York. Charles C. Thomas, Publishers, 220 East Monroe Street, Springfield, Illinois, U.S.A. Pp. 178, cloth, Price $4.00 postpaid.

In this treatise Dr. Landsteiner attempts to give as accurately as possible the present concept of the chemistry of serological reactions. The word "attempt" is used advisedly, for, as frequently inferred by the author, so much more could be learned concerning serological reactions if organic chemistry were sufficiently advanced to permit analysis of proteins, antibodies, antigens and related substances. One cannot help but feel a deep respect for the author as he has a thorough command of his subject and has included a bibliography of 1164 references from the world literature including his own numerous contributions. The material is very compact—no words are wasted. Where detailed explanations are thought advisable the reader is given several references. Theories and hypotheses are briefly used to explain certain discrepancies in experimental results and necessarily so inasmuch as our knowledge of the organic chemistry involved is relatively meager. The work is technical insofar as a thorough understanding of all terms used in serological reactions and at least a basic knowledge of organic chemistry are essential.

The book is divided into six chapters to include 1. Introductory Remarks; 2. The Serological Specificity of Proteins; 3. The Specificity of Cell Antigens; 4. The Specificity of Antibodies; 5. Artificial Conjugated Antigens; Serological Reactions with Simple Chemical Compounds; and 6. Chemical Investigations on Specific Cell Substances; Carbohydrates, Lipoids.

This monograph would be of distinct value to those doing any form of bacteriological or serological work, especially in the experimental field as many gaps in our knowledge of the subject are pointed out leaving plenty of room for further investigation. It is highly instructive and entertaining for those particularly interested in this field but who are not carrying on investigative work themselves. Every physician using immunotherapy could gain valuable additional information concerning the reasons for his treatment insofar as our present knowledge of them will permit. Points of value can be had for those in the investigative field of medico-legal work.

ABSTRACTS

A RAPID METHOD FOR PREPARING ANTIGENS FOR THE WASSERMANN REACTION: Charles A. Hunter, Ph.D., Jour. Lab. and Clin. Med., Vol. 21:4, January, 1936.

By the use of the Soxhlet apparatus, the author has given us a method of extracting the lipoids from beef heart which required only from eight to ten hours as compared with the fifteen days of Kolmer's method. Antigens thus prepared are of high antigenic titer, and have low anticomplimentary and hemolytic units. The antigens also have good specificity by comparison with Kahn's precipitation test on at least 50,000 serums.

A RAPID SLIDE TEST FOR THE SEROLOGIC DIAGNOSIS OF TYPHOID AND PARATYPHOID FEVERS: Henry Welch, Ph.D., and C. A. Stuart, Ph.D., Jour. Lab. and Clin. Med., Vol. 21:4, January, 1936.

In this rapid slide test concentrated antigens and undiluted serums are used. The procedure takes only a very few minutes and the results have proved to be as accurate and as specific as the macroscopic tube test with which it was compared. The only materials required are a standard dropper pipette, glass slides, wire loop for preparing wax rings which are made of paraffin and petrolatum.

PIONEERS

LOUIS PASTEUR

(1822-1895)

A chemist whose name is intimately linked with medicine because of his discovery of preventive vaccination, particularly of hydrophobia.

MONONUCLEOSIS, INFECTIOUS: I. Davidsohn, Am. J. Dis. Child. 49:1222, 1935.

The following rapid method for the heterophile antibody test gives final readings in four hours:

The serum dilutions range from 1:2.5 to 1:5,120, or higher if necessary. The quantity of dilution used is 0.25 cc. One-tenth cubic centimeter of a 2% suspension of sheep cells is added. Narrow test tubes, measuring 75 mm. in length and 12 mm. in the external diameter, are used in order to make possible the reading of the results with a microscope. The test tubes are shaken and placed in a water-bath at 38° C. for one hour and then in an ice-box for one hour. At the end of two hours, the test tubes are shaken until the sediment is suspended, and the results interpreted as +++ and ++ are estimated with the naked eye. The result read as + can be fairly accurately estimated with the naked eye, but a finer, more accurate reading is obtained by placing the test tube flat on its side on the stage of the microscope and looking at the cell suspension with the low power of the objective. The titers obtained with the microscope are from one to two dilutions higher than those read with the naked eye.